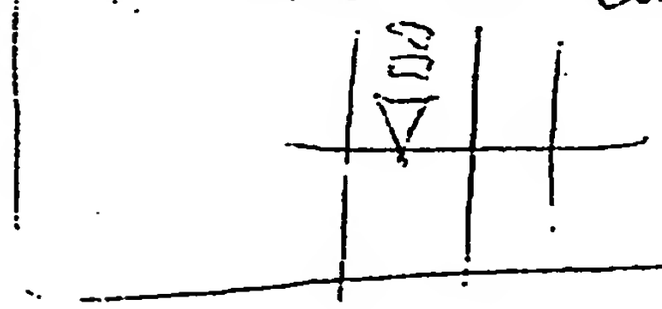
Significant
* select the return
① statistics
② Regression wizard
③ Sigmoidal → parameter
④ Logistic 4. parameter
if higher parameter does not work,
use lower one (3)

⑤ parameters : First empty
need in report ☑
create new graph ☑

↓

Finish

* file or column



* folder list can name change
Rt click : Edit click
→ type new name

① IgG 1mg/ml 50ul 1hr
② Block 200ul 1% BSA
③ Enz anti-IgG 50ul
Substrate 50ul

50ul

IgG (4g dilute)

1:10 1:10 1:100 1:100 1:500 1:500 1:1000 1:1000

	1	2	3	4	5	6	17	8	9	10	11	12
A	Blank	IgG	1:10	1:10	1:100							1:1000
B		1:10	1:10									"
C		1:10	1:10									1:2000
D		1:10	1:10									"
E		1:10										1:4000
F		1:10										"
G												
H												

(5) (ml)
1 ~ 1000 ml
0.1 ~ 100
0.01 ~ 10
0.005 ~ 5ml
(4.995)

2ml + 2ml
0.1% BSA-TBS

12x 0.05ml (1ml) PBS
05
10

1:10 1ml 0.1ml + 0.9ml PBS
1:100 1ml 10ul + 0.99 ml
1:500 1ml 0.2ml + 0.8ml
1:1000 1ml 0.1ml (100) + 0.9ml

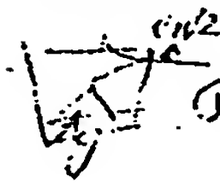
0.9ml

0.2ml 1.8ml

1ml = 1000ul
0.2ml = 200ul

	1	2	3	4	5	6	7	8	9	10	11	12
A	3kof											
B												
C												
D												
E												
F												
G												
H												

1 well = 1 sample



1) Antigen — 1hr to overnight in bicarbonate buffer of H.S.O (sticky)

2) block w add blocking reagent 1% BSA and PBS (phosphate buffered saline) ↳ 1 hr wait

3) Add Antibody (human serum) ↳ 1 hr wait

4) block.

5) Add indirect enzyme conjugated Ab ↳ 1 hr wait

6) Wash → Add substrate

Ag { 1:100 (bicarbonate) ↓ 20ul + 2ml
1:500 (") ↓ 200ul + 0.8ml
1:1000 (") 200 ul of (1:100) + 1.8 ml bicarbonate
1:2000 (") 0.05ml (50ul of 1:100) + 1.0ml bicarbonate

human IgG in bicarb

1:100 1:100 1:500 1:500 1:1000 1:1000 1:2000 1:2000

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank											
B												
C												
D												
E												
F												
G												
H												

1:100 → 1:2000 → 1:4000 → NOT H → 1:2000 → NOT H

Ag
* 1:100 → .1 x 0.0
.01 → 1 ml
20ul + 2ml
12 x 0.05 ml = .60ml
(12 .01 .60)

* 1:500 1:5 dil = 1:500 0.1ml x 0.X
0.2ml x 0.8

1:1000 dil 0.1ml + 0.9ml (0.2ml + 1.8)
1:2000 dil ⇒ 1:20 dilen 1 → 20 or .1 → 2.0

.1 → 2.0 ml
0.05 → 1ml

0.05 → 1.0 ml
(1:20)

① Eliza : basic concept.

Ag (thyroglobin)

Block + Pt's Serum (1:50 dilution
(1% Bovine serum albumin) (Anti Ab to thyroglobin) { 1ml → 50ml
0.1ml → 5ml
0.01ml → 0.5ml
(10ul → 500ul)

Ig (thyroglobin)
(Ag)

Anti Ab

(goat anti human IgG:
Ap: Alkaline phosphatase
(human)

(2)

1:56
Alk phosph anti human
1:56
substr

1000 µg/ml in PBS

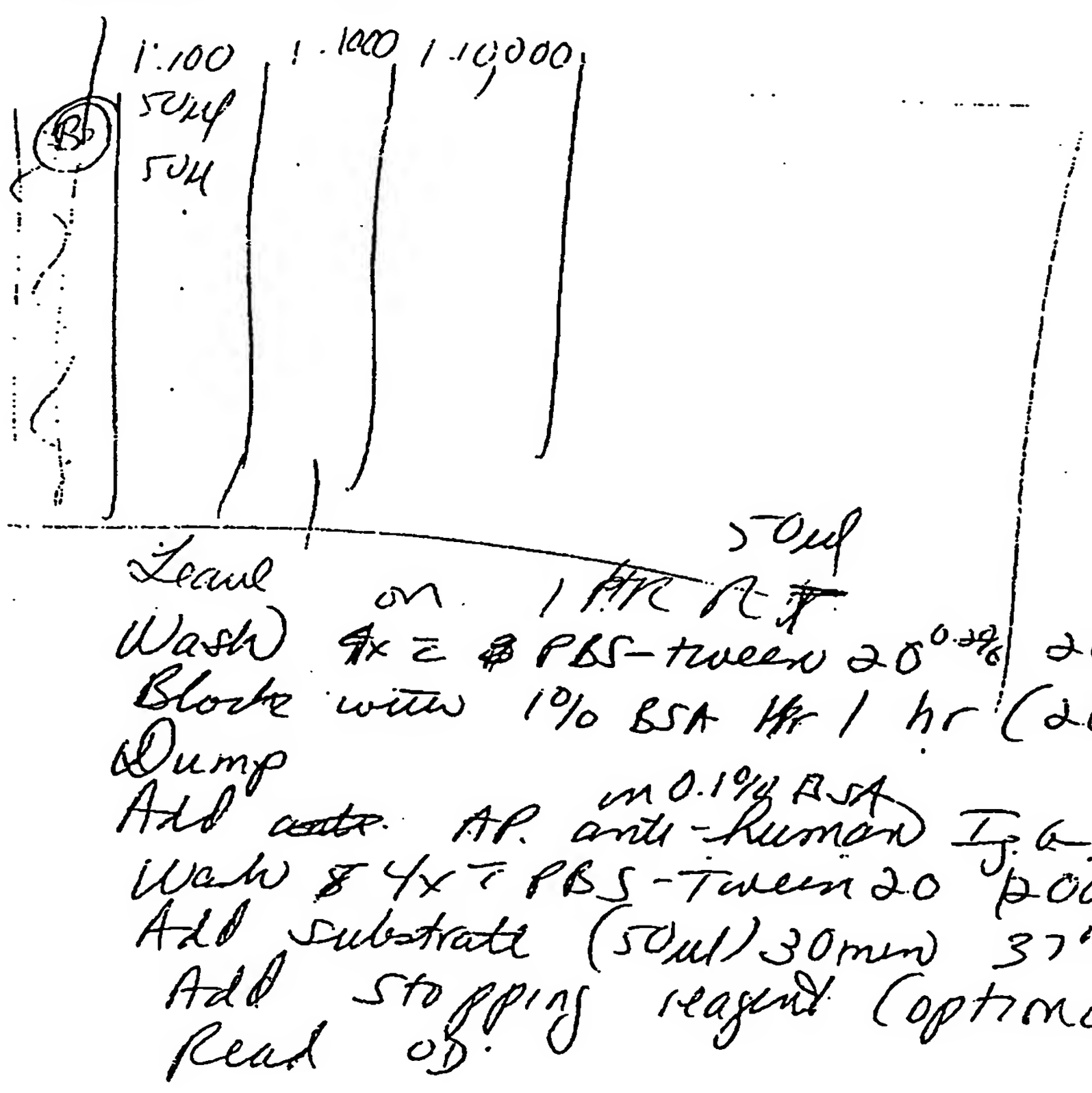
1: 100

1: 1000

1: 10,000

= Bicarbonate buffer.

Bicarb STD



Load on 1 hr RT
Wash 4x = PBS-tween 20 0.2% 200µl
Block with 1% BSA Hr / hr (200µl)
Dump
Add ~~conte~~ AP anti-human IgG (50µl) /hr
in 0.1% BSA
Wash 4x PBS-Tween 20 (200µl)
Add Substrate (50µl) 30min 37°C
Add stopping reagent (optional)
Read OD

1ml : 199 ul PBS

① anti-CD Ab ( ) mg/ml
→ dilute 1:200 in PBS [No BSA, No Serum],
place 30 ul in each well for stimulation.

② Incubate 1 hr at 37°C or
Overnight at 4°C (refrigerator)

③ Wash cells 2x with 200 ul PBS - tap out on paper towel (should be sterile)

④ * No stim cells at least 1 away from stim

Stim		N
●	○	○
●	○	○
●	○	○
●	○	○

⑤ 200,000 cells/well in 200 ul
(= 1 x 10⁶/ml)

⑥ for flow, Centrifuge in regular tubes, put supernatant into eppendorf.

⑦ Collect S/N at 24 hrs, Centrifuge in microfuge and place in a fresh tube

⑧ Assay by ELISA immediately or
freeze S/N ≤ -20°C

if multiple ELISA; Aliquot S/N.

— Read ELISA protocol ahead of time
How much sample do you need?

IL-10 1:10 → 100 ul

~~PHA~~ (2 ug/ml) ± αCD3 (+ αCD3, − αCD3)] compare

⊛ R & D Systems
DO NOT USE BIOSOURCE (?)

PBMC ← Stim Anti CD3

IL-2 ⇓ (IFNγ) Th1
IL-10 ⇑

Rx (10 ml women
10 preg women

αCD Ab 2 ng/ml (1 mg/ml)

anti CD3 10 ug/ml

in PBS (NO BSA, NO SERUM)

① dilute 1:200, place 30 μl in each well for Stimulation

~~(anti CD3 → (... PBMC)~~

② incubate (1 hr at 37°C) or overnight at 4°C (refrig.) (sterile)

③ wash wells 2x with 200 μl PBS - tap out on paper towel.

* no stim wells at least 1 away from stim

S NS

④ 200,000 cells/well in 200 μl = 1×10⁶/ml) → 24 hr incubation (37°C CO₂)

(5a) for flow, c. fuge in regular tubes, put S/N into eppendorf

⑤ collect S/N at 24 hrs centrifuge in microfuge 5 min and place in a fresh tube.
→ collect the supernatant ĉ Eppendorf pipette

⑥ assay by ELISA immediately or

freeze S/N ≤ -20°C
if multiple ELISA's, aliquot S/N

- read ELISA protocol ahead of time
HOW MUCH SAMPLE DO YOU NEED?

#C-10

Coulter Epics (Turn On)

① Computer Power On → (wait 20 min)

② 맨 왼쪽 뚜껑 (가운데 검은 box) - orange line 검사
- waste box check - 1/2 이상이면 dump
- (2 white bottle - dry 해야
  (2 Transparent bottle - 2/3 이하 해야
- Orange 읽어서 → (기록)

③ Panel → select → start up click & okay click

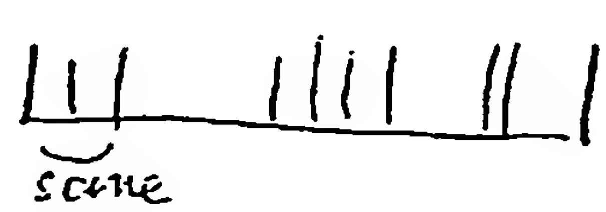
④ 뚜껑 맨 왼쪽 Run 누르고 green blink 되면
open the door (왼쪽 긴 drawer)
→ Isofluid 채우고
bottom 2-3회 눌러 bubble 없는지 check

⑤ 화면에 Error message - click
clear Error - click

⑥ Carrossal 에 3개 tube
① Water 1 ml 넣고
② F-check : 10 drop
③ F-Set : 10 drop

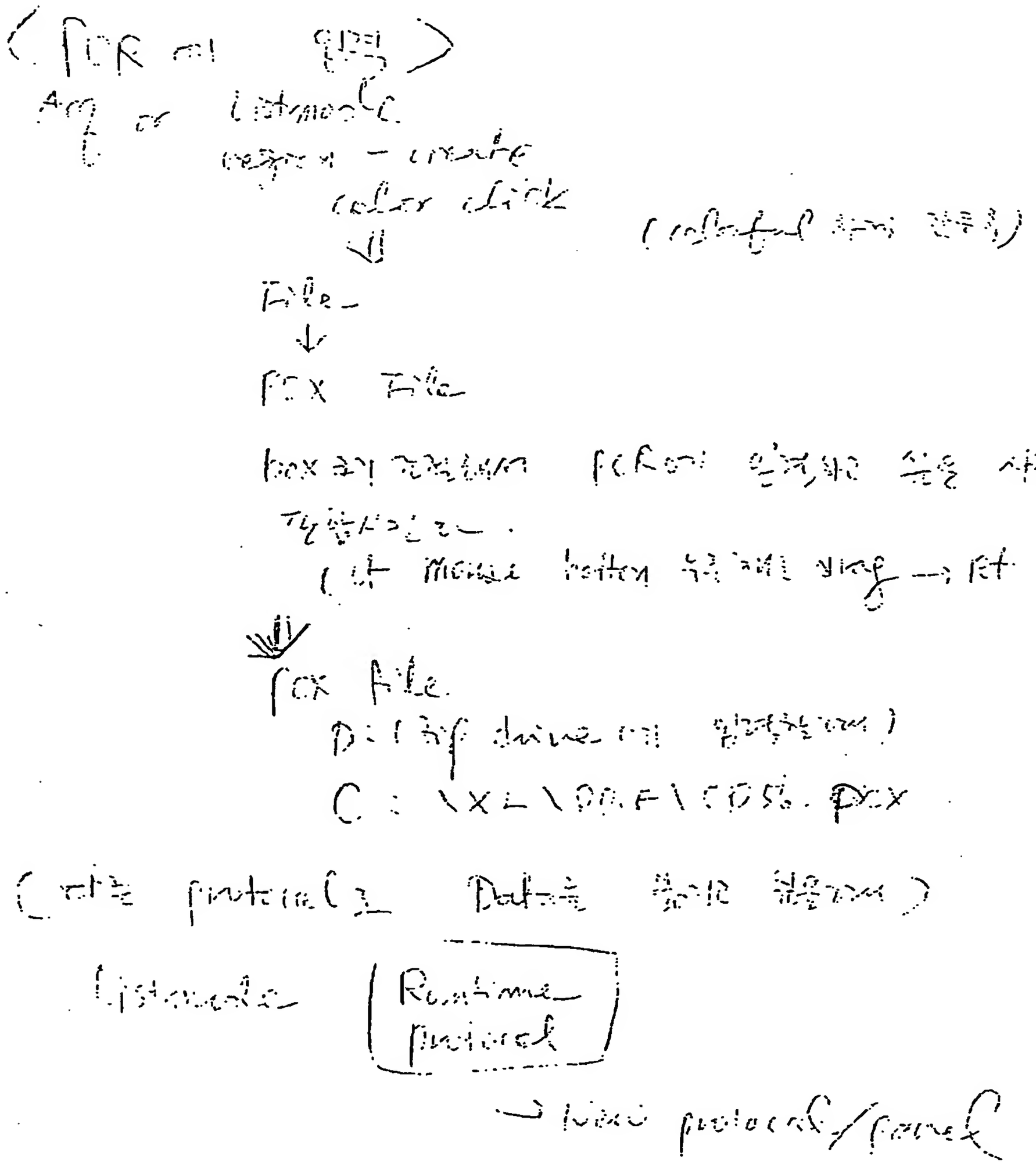
⑦ 뚜껑 Run 눌러 initialization orange 불이 안 들어

⑧ Insert tube 화면 뜨면
okay click → 5-7분 wait

⑨ Flow-check 경우 : 

HPCV = CV
MnFX = Mean CH
Flow-Set "
MnX = Peak CH Copy 함

⑩ Protocol → Select.



Shutdown:
① water
② ~~sw~~ bleach
③ water
④ water
} about 1ml

Panel
→ Select
→ shut down
tube ← 넣는다
→ Run (tube 8-10 min) 끝나면 Run botton → green → Push botton
↳ it will be blinking
(Manual clamp)
put the water
black tube 에 2X (양쪽 다 채움) ← in manual tube
→ I can open the door
green + blinking (앞으로 약간 기울이면서 연다)
→ take out ~~test~~ tube
→ black tube 놓음
→ 다른 black tube 넣음
→ test tube 다시 넣음

Auto mode procedure ①. ②
put 2 tube water
carousel 에 넣음
→ Auto / Reverse → 동시에 누름